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AVIAN CELL-MEDIATED IMMUNE RESPONSE TO DROUGHT

J. M. FAIR^{1,2} AND S. J. WHITAKER¹

ABSTRACT.—Regional droughts have far-reaching, substantial, and easily recognizable impacts on populations and the environment. One component of these impacts that is not widely recognized is impairment of immune function by drought-related physiological stress. We studied cell-mediated immune function of cavity-nesting Western Bluebirds (*Sialia mexicana*), Ash-throated Flycatchers (*Myiarchus cinerascens*), and Violet-green Swallows (*Tachycineta thalassina*) at Los Alamos, New Mexico. There was a dramatic decrease in the cell-mediated immune responsiveness of developing nestlings associated with unusually dry conditions. Adult Western Bluebirds captured in 2002 weighed 7% less than in all previous years and average clutch size for all three species was reduced by 21% in 2002. Nestling body mass was also reduced for flycatcher and bluebird nestlings in 2002 compared to all other years. Survival to fledging age was lower overall during the drought years of 2000–2002 compared to the first 3 years of the study. Received 17 April 2006. Accepted 14 February 2008.

Regional droughts have far-reaching, substantial, and easily recognizable impacts on populations (George et al. 1992) and the environment (Allen and Breshears 1998). One component of these impacts that is not widely recognized is impairment of immune function by drought-related physiological stress. There is ample evidence that stress affects immune system function (Apanius 1998), but a reduction in immunity has not been documented directly with drought conditions. One consequence, global warming, as frequency and severity of drought increases, may be increased vulnerability of populations to disease accompanied by more frequent disease epidemics (Epstein 2001; Harvell et al. 2002; Møller and Erritzøe 2003; Shaman et al. 2003, 2005). Rising temperatures could expand the distribution of vector-borne pathogens transmitted by arthropods, including mosquitoes, sand flies, midges, and ticks (Shope 1992, Epstein and Defilippo 2001, Shaman et al. 2005); many arthropod-borne disease epidemics are associated with droughts (Epstein and Defilippo 2001).

The drought of 2000–2002 in the southwestern United States, although not unprecedented (Allen and Breshears 1998), was one of the most severe in 50 years. We assessed cell-mediated immune response from 1997 to 2002 using a phytohaemagglutinin (PHA) injection in nestlings of three species of cavity-

nesting birds: Ash-throated Flycatcher (*Myiarchus cinerascens*), Western Bluebird (*Sialia mexicana*), and Violet-green Swallow (*Tachycineta thalassina*). PHA response has been associated with body condition and survival (Hörak et al. 1999, Alonso-Alvarez and Tella 2001). PHA injected for localized *in vivo* inflammatory response in birds has long been used to measure cell-mediated immunity (Staddecker et al. 1977, Lamont and Smyth 1984) and has been found to not impose additional stress or survival costs (Merino et al. 1999, Smits and Williams 1999). PHA has been shown to increase energy expenditure and food intake (Martin et al. 2003, Barbosa and Moreno 2004). Martin et al. (2006) found that PHA-induced swelling is related to heightened immune cell activity in House Sparrows (*Passer domesticus*) and that PHA-induced swelling is a tradeoff with other physiological functions.

Cell-mediated immunity is particularly important during the breeding season, because the likelihood of injury during sexual competition is high and cell-mediated immunity is essential for healing wounds and resisting infection (Zuk and Johnsen 1998). Our objectives were to: (1) compare three similar species in relation to nestling cell-mediated immune response, condition, and survival; and (2) examine the effects of severe drought on both nestlings and adults during the breeding season.

METHODS

Study Area.—This study was conducted at Los Alamos National Laboratory (LANL) in

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north-central New Mexico. The 111-km² facility is on the Pajarito Plateau and consists of a series of relatively narrow mesas separated by deep, steep-sided canyons that extend west to east-southeast along an elevation gradient from the Jemez Mountains to the Rio Grande. Vegetation community types range from open ponderosa pine (*Pinus ponderosa*) forest to piñon-juniper (*P. edulis*, *Juniperus osteosperma*) woodland. We placed 433 bluebird boxes on LANL property during winter 1997. Nest boxes were placed ~2 m above ground level on trees and spaced ~50–75 m apart.

All nest boxes were visited every 2 weeks starting in May 1997; nests with eggs were considered active and visited every 2 days until the first eggs hatched (day = 0). Nests were visited again on days 3, 10, 15 and 16. Ten percent of the Western Bluebird pairs had second broods, which did not differ between years and were not included in the analysis. All nests included in the analysis were new pairs not previously in the study. Each nestling was handled for <8 min/visit in accordance with the Guidelines for the Use of Wild Birds in Research (Gaunt and Oring 1997).

Measurements.—All nestlings in selected nests were injected intradermally on day 15 in the wing-web with either 0.05 ml (1.0 mg/ml) PHA in phosphate-buffered saline (PBS) (right side) or 0.05 ml PBS only (left side). This date was chosen, as it was latest date before fledging when this could be completed. The amount of swelling in the wing-web 24 hrs after inoculation was measured with a pocket dial gauge micrometer (L.S. Starrett® #1010, Athol, MA, USA) to the nearest 0.001 mm by the same person. The lower variance within individuals for PHA measurements using the left wings of Western Bluebirds and Ash-throated Flycatchers in 2002 with wing webs not injected with PBS (post 24 hrs) was derived from a one-way ANOVA using the GLM (general linear model) in SAS (SAS Institute 1998). Our estimates of individual variance, consistent with Lessells and Boag (1987), used the correlation coefficient calculated using the variance component of the data set. The variability of measuring PHA with just one observer was estimated to be 0.94 and associated $F_{1,81}$ ratio of 1127. A PHA index (Fair et al. 1999) to compare species

was computed as the thickness of the PHA-inoculated wing-web minus the thickness of the opposite wing-web and standardized by the average wing thickness before inoculation. All PHA measurements were made by one person (JMF).

Nestlings were weighed at 15 days and survival of 864 nestlings was calculated up to the maximum of 14 days of age after hatching over 6 years. The length of the right ninth primary was measured with a ruler to the nearest mm using the flattened wing method (Svensson 1984). The left and right tarsi were measured with digital calipers to the nearest 0.1 mm. All birds were weighed using a digital balance to the nearest 0.01 g. Predation was relatively low with most known dead birds being found in the nest (Fair et al. 2003).

Probability of fledging for all three species after 14 days was 68% for all years combined. PHA-induced wing web swelling was measured for 225 Western Bluebird (WEBL), 40 Ash-throated Flycatcher (ATFL), and 33 Violet-green Swallow (VGSW) chicks. The majority of the flycatchers and swallows were measured for PHA in 2001 and 2002. Three hundred flycatchers were additionally measured for morphological parameters. Approximately 70- μ l of blood was collected on day 15 of age from the brachial vein of the wing in heparinized microcapillary tubes, which were kept in a cooler. Heparinized capillary tubes were spun for 10 min in a microcapillary centrifuge within 1 hr of collection. Hematocrit estimates from the blood collected were obtained directly using a microhematocrit reader.

Data Analysis.—We used SAS statistical software (SAS Institute 1987) for all analyses. PHA measurements were heteroscedastic between years ($F = 5.50$, $P < 0.0003$, Levene's Test), the data within nests were not independent, and we used the means of the PHA measurements for each nest in a nonparametric Kruskal-Wallis Test. Weight data were also not independent but had similar variances between years and we used a mixed general linear model (PROC MIXED). The nest box was included as a random factor to account for non-independence among young within a brood and fixed factors included nestling variables of body mass, species, and year. Comparisons of bluebird and flycatcher nestling

survival rates were analyzed using the log-rank procedure LIFETEST (SAS Institute 1987), which allows for right censoring of data points where the failure times to the right are missing. The log-rank procedure LIFETEST computes nonparametric estimates of the survivor function and rank tests for association of the response variable with other variables; it is more robust than the Wilcoxon statistic in detecting differences between groups.

RESULTS

Precipitation for this region was 25% below average during 2000 and 2001 and 65% below average through summer (Aug 2002). All three species had a similar reduction in the PHA-induced wing-web swelling response in 2001 and a more pronounced reduction in 2002 compared to the first 3 years of the study (WEBL, $\chi^2 = 40.3$ df = 4, $P < 0.0001$; ATFL, $\chi^2 = 10.2$, df = 3, $P = 0.0002$; VGSW, $\chi^2 = 13.4$, df = 1, $P = 0.0003$, Kruskal-Wallis) (Fig. 1A). Response to PHA increased with annual precipitation for all three species (WEBL [$n = 225$], $r_s = 0.23$, $P = 0.003$; ATFL [$n = 40$], $r_s = 0.73$, $P < 0.001$; VGSW [$n = 33$, 2 yrs] $r_s = 0.42$, $P = 0.01$, Pearson Rank Correlation) (Fig. 1B). Western Bluebird and Ash-throated Flycatcher did not differ in response to PHA for the first 3 years of the study. However, Western Bluebird had a higher response in 2001 and 2002 to PHA than Ash-throated Flycatcher and Violet-green Swallow ($\chi^2 = 8.8$, df = 2, $P = 0.01$; $\chi^2 = 14.3$, df = 2, $P = 0.0008$), respectively. PHA response was not related to clutch size in any of the three species for any year except for Western Bluebird where birds with smaller clutch sizes (2 and 3) had lower responses to PHA than those with larger clutches (4 and 5) ($\chi^2 = 3.7$, df = 1, $P = 0.05$). One hundred and thirty-four birds received an antigenic challenge when they were 5 days of age; we tested for the any correlations between PHA response and the three antigens and control, and found no relationship ($F = 0.98$, df = 4 and 133, $P = 0.43$). This was not a confounding factor in the analysis.

Nestling body mass (g) was reduced for flycatcher and bluebird nestlings which weighed less in 2002 compared to all other years ($F = 18.02$, df = 3 and 36, $P < 0.0001$ $F = 31.02$,

df = 4 and 220, $P < 0.0001$, respectively). The average body mass for Ash-throated Flycatcher was significantly lower in 2002 than all other years (LSD test) and was significantly lower for Western Bluebird in both 2001 and 2002 than in other years. Violet-green Swallows did not have previous years for comparison of body mass. Similarly, the right wing length of the ninth primary was significantly shorter in 2001 and 2002 for Western Bluebird ($F = 14.3$, df = 4 and 220, $P < 0.0001$), Ash-throated Flycatcher ($F = 12.1$, df = 4 and 87, $P < 0.0001$) and, in 2002, for Violet-green Swallow ($F = 7.5$, df = 1 and 31, $P < 0.01$). Length of the right tarsus did not differ between any years for Western Bluebird ($F = 0.62$, df = 4 and 220, $P < 0.0001$).

Survival to fledging age was not lower in 2002 ($\chi^2 = 54.3$, df = 5, $P < 0.0001$, log rank test) than in the previous 2 years, but was lower overall during the drought years of 2000–2002 compared to the first 3 years of the study (Fig. 2). Percent of eggs that hatched per clutch did vary between years for Western Bluebird with a reduced clutch size in 2002 from all other years ($F = 12.86$, df = 5 and 632, $P < 0.0001$). This was also similar for Ash-throated Flycatcher ($F = 10.38$, df = 5 and 223, $P < 0.0001$) and for Violet-green Swallow in 2002 ($F = 5.47$, df = 2 and 26, $P = 0.01$). Average clutch size for all three species was reduced by 21% in 2002. There was variation in hematocrit between years ($F = 3.32$, df = 5 and 128, $P = 0.007$) in adult Western Bluebirds that were captured, bled and banded ($n = 133$); but there was no clear pattern for year differences. There was also variability in mass (g) for adult bluebirds between years (2002, $\bar{x} \pm \text{SE} = 23.9 \pm 0.87$ g; other years 25.8 ± 0.44 g) with adults captured in 2002 weighing 7% less than in all previous years ($F = 4.50$, df = 5 and 112, $P = 0.0009$).

DISCUSSION

There was a dramatic decrease in the PHA-induced wing-web swelling in nestlings associated with unusually dry conditions. Adult Western Bluebirds captured in 2002 weighed 7% less than in all previous years. Average body mass was also reduced for flycatcher and bluebird nestlings, which weighed less in

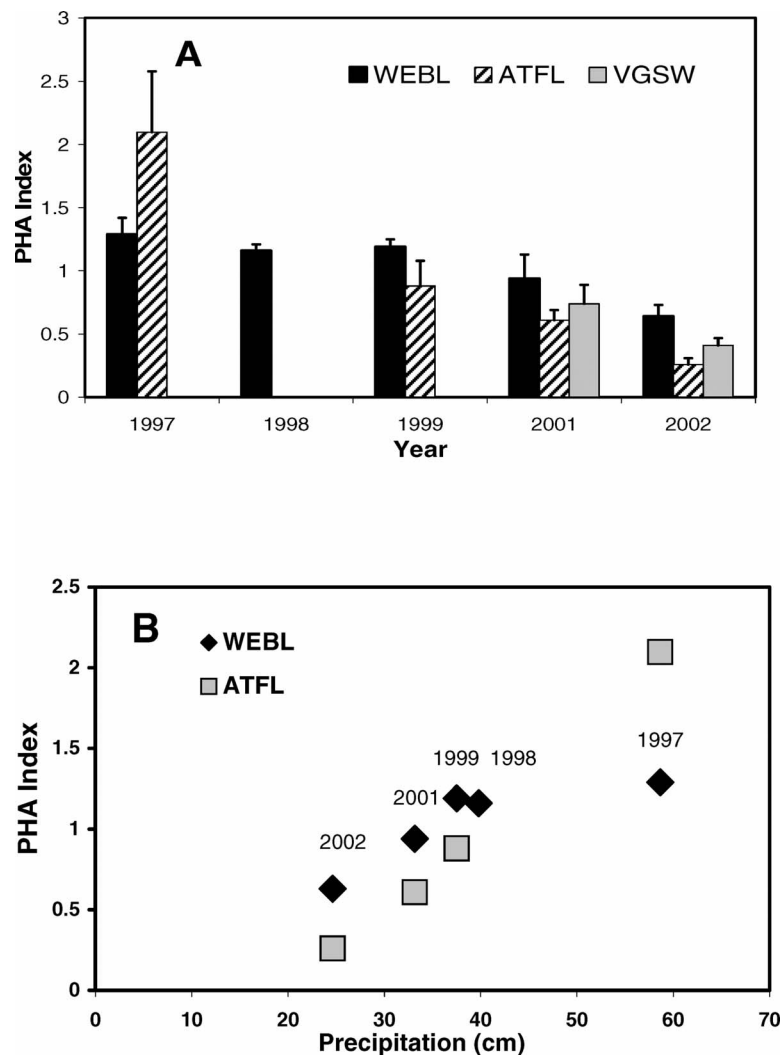


FIG. 1. (A). Cell-mediated response to PHA was significantly reduced in 2002 from all other years (PHA index [mean \pm SE]). Morphological and immune data were not collected during 2000 due a large-scale fire adjacent to the study area in May 2000. (B). Cell-mediated response to PHA for annual average precipitation from 1997 to 2002.

2002 compared to all other years, indicating nutritional stress was potentially related to drought conditions. Survival to fledging age in Western Bluebird nestlings was not lower in 2002 than in the previous 2 years, but was lower overall during the drought years of 2000–2002 compared to the first 3 years of the study (Table 1). Hematocrit did not provide additional information on impacts of drought on condition due to variability between individuals and years.

There is no direct published evidence for

effects of drought on cell-mediated response, but numerous studies document the cell-mediated immune function is strongly correlated with nestling body mass (Saino et al. 1997, Christe et al. 1998, Tella et al. 2001, Westneat et al. 2004), availability and quality of food (Lochmiller et al. 1993, Birkhead et al. 1998, Gonzales et al. 1999, Hoi-Leitner et al. 2001, Lifjeld et al. 2002) and the overall environment (Brinkhof et al. 1999, Tella et al. 2000). Lifjeld et al. (2002) found that cell-mediated immune response is affected by weather con-

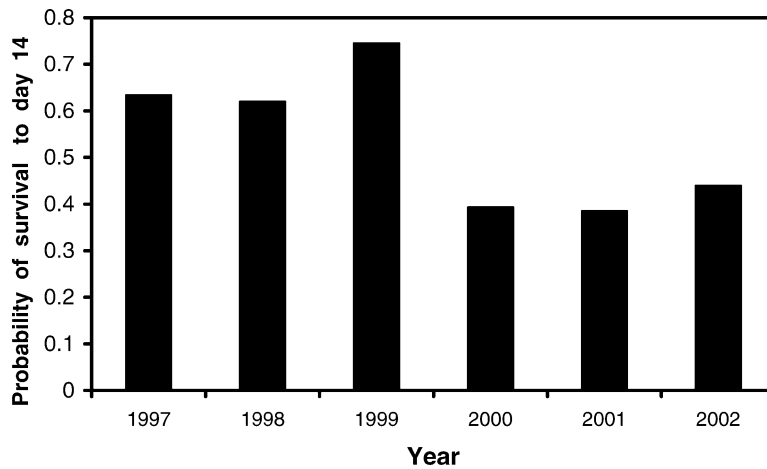


FIG. 2. Probability of nestling survival to day 14, before fledging, for 1997–2002.

ditions in particular, and that PHA response is influenced by short-term fluctuations in energy balance. The PHA response in this study was measured over the entire summer and the measurements should include daily variation during the breeding season. We demonstrate wide variation in PHA between years that coincides with a significant drought, but do not have food limitation data to identify the exact relationship. It is also not clearly understood how much variation in PHA response is heritable, as there has been conflicting evidence (Brinkhof et al. 1999, Tella et al. 2000, Martin et al. 2006). Other environment conditions such as increased exposure to predators have also been shown to decrease the avian cell-mediated response (Navarro et al. 2004).

The mostly likely mechanism for reduction in cell-mediated immune response, reduced weight, and nestling survival would be food availability and parental effort. There has been relatively little research documenting impacts of drought on arthropod communities, but one study experimentally showed that drought negatively impacted farmland arthropod communities (Frampton et al. 2000). Studies on the impacts of drought on arthropods or other prey in relation to parental effort and development would help in understanding the exact mechanism involved in impacts of severe drought on birds and immune function.

One of our most significant findings was that clutch size for all three species was reduced by 21% in 2002—a result that follows

TABLE 1. Average individual and nest characteristics for three cavity-nesting species for 1997–1999 (average precipitation) and 2000–2002 (below average precipitation).

Species and year	Body mass, g	Tarsus length, mm	Survival to day 14, %	PHA Index (n)	Clutch size	Hatch date	Percent hatch	Number of individuals for PHA	Number of nests
Ash-throated Flycatcher									
1997–1999	25.6	26.3	50.7	1.7 (10)	3.9	175.8	75.7	10	53
2000–2002	25.2	25.5	23.0	0.51 (20)	3.4	170.8	67.1	30	50
Violet-green Swallow									
1997–1999									
2000–2002	17.5	13.6	44.8	0.51 (33)	3.3	183.3	70.2	33	30
Western Bluebird									
1997–1999	26.5	23.0	74.5	1.2 (197)	4.5	165.4	70.6	197	170
2000–2002	22.7	22.8	53.2	0.76 (28)	4.1	164.2	72.8	28	90

Tella et al. (2000) who found that cell-mediated immune response was higher in nestlings from larger clutches. However, this does not follow the studies of Hōrak et al. (1999) and Ilmonen et al. (2003) who found an increase in brood size reduced the cell-mediated immune response. We found no relationship between timing of breeding during the season (hatch date) and response to PHA. Many of the tradeoffs between brood size, development, parental effort, and food availability remain to be understood, but the impact of extreme environmental conditions can be measured in immune function. Martin et al. (2006) noted that PHA-induced swelling does not appear to be an unambiguous index of T-cell-mediated immunity, but rather a multifaceted index of cutaneous immune activity that may be affected by the physiological state of the individual as well as the life-history traits of the species. Our study documents a decrease in PHA-induced swelling over several years on the same day of nestling development within the same species that is associated with a reduction in precipitation and nestling mass and survival.

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